Micronuclei in Exfoliated Bladder Cells among Individuals Chronically Exposed to Arsenic in Drinking Water

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Abstract
Inorganic arsenic is an established cause of lung and skin cancer. Epidemiological evidence from Taiwan suggests that arsenic causes more fatal internal cancers, with the highest relative risks reported for bladder cancer. We conducted a cross-sectional biomarker study in a Chilean male population chronically exposed to high (70 subjects) and low (55 subjects) arsenic levels in their drinking water (average concentrations, 600 and 15 μg/liter, respectively). A fluorescent version of the exfoliated bladder cell micronucleus (MN) assay was used employing fluorescence in situ hybridization with a centromere probe to identify the presence (MN+) or absence (MN−) of whole chromosomes within micronuclei, thereby determining the mechanism of arsenic-induced genotoxicity in vivo. We divided the study population into quintiles by urinary arsenic levels and found an exposure-dependent increase in micronucleated cell prevalence in quintiles 2–4 (urinary arsenic, 54–729 μg/liter). The largest increase appeared when quintile 4 was compared to quintile 1 [prevalence ratio, 3.8; 95% confidence interval (CI), 1.9–6.6]. The prevalence of MN+ increased to 3.1-fold in quintile 4 (95% CI, 1.4–6.6), and the prevalence of MN− increased to 7.5-fold in quintile 3 (95% CI, 2.8–20.3), suggesting that chromosome breakage was the major cause of MN formation. Prevalences of total MN, MN+, and MN− returned to baseline levels in quintile 5 (urinary arsenic, 729–1694 μg/liter), perhaps due to cytostasis or cytotoxicity. These results add additional weight to the hypothesis that ingesting arsenic-contaminated water enhances bladder cancer risk and suggest that arsenic induces genetic damage to bladder cells at drinking water levels close to the current United States Maximum Contaminant Level of 50 μg/liter for arsenic.

Introduction
Arsenic is a naturally occurring metalloid element ubiquitous throughout the earth’s crust. An acute dose of In-As8 can cause severe organ damage and death, and chronic poisoning can cause less specific symptoms such as weakness, fatigue, absent motivation, anorexia, and weight and hair loss (1). Chronic exposure to In-As is an established cause of lung cancer via inhalation and skin cancer via ingestion (2). Recent evidence suggests that ingestion of In-As may also cause more fatal internal cancers, including bladder cancer (3, 4).

Previously, we conducted a small biomarker study (18 matched pairs) to investigate the relationship between chronic ingestion of In-As and frequency of MNC (5). The results showed a positive exposure-dependent increase of bladder cell MN frequency with urinary As levels, suggesting that chronic ingestion of In-As may have a genotoxic effect on the bladder epithelium. To further test these findings, we conducted a larger cross-sectional biomarker study in a population in Chile chronically exposed to high As levels in drinking water.

Exfoliated bladder cells are epithelial cells sloughed from the surface of the genitourinary tract that turn over in the bladder every 1–3 weeks (6). Because epithelial cells are derived from basal cells, recent genetic damage to the basal layer of the bladder could be reflected in the presence of micronucleated exfoliated cells. MN are extranuclear bodies in the cytoplasm of a cell that form during cell division whenacentric fragments or whole chromosomes are left behind the main nucleus at telophase. An increase in the prevalence of MN within a population of cells is an indication that chromosome damage has occurred as a result of exposure to an agent that caused either a clastogenic or aneuploidaligeneffect (7). Arsenic has been shown to have both clastogenic and aneuploidaligene properties in human lymphocytes using both the MN assay and chromosome-specific probes in vitro (8–10). Vega et al. (11) recently evaluated the aneuploidaligenepotential of As in vitro and found a dose-related increase in hyperploid lymphocytes. To determine whether As is acting primarily as an aneuploidaligenor a clastogen in vivo, we used a fluorescent version of the MN assay that employs FISH to identify the presence (MN+) or absence (MN−) of centromeric probe within a MN. MN positive for the centromeric probe (MN+) are thought to contain a whole chromosome and to have arisen via an aneuploidaligenevent (e.g., spindle disturbances), while micronuclei negative for the centromeric probe (MN−) are

* The abbreviations used are: In-As, inorganic arsenic; MN, micronucleus/micronuclei; FISH, fluorescence in situ hybridization; MMA, methylarsonic acid; DMA, dimethylarsinic acid; MNC, micronucleated bladder cell; CI, confidence interval; PR, prevalence ratio; As, arsenic.
assumed to contain only acenetic fragments of DNA and to have arisen via a clastogenic event (e.g., chromosome breakage). Like antigenchecore antibody staining (8), FISH staining can be used to understand the mechanism of action of the environmental carcinogen. Unlike the antigenchecore assay, which is limited to viable cells such as lymphocytes, FISH can also be used in exfoliated bladder cells that originate from epithelial tissues, the site of As-induced cancers. In this study, we used the fluorescent MN assay to: (a) confirm whether chronic ingestion of high levels of In-As is associated with a detectable increase in the prevalence of exfoliated bladder cell MN in a large study population; (b) determine the mechanism of As-induced MN formation in the target tissue based on the induction of Mn⁺ and Mn⁻; and (c) examine the dose-response relationship between As exposure and MN formation in bladder tissue.

Materials and Methods

Selection of Study Subjects. Study subjects included residents from two towns in Northern Chile who drank water from sources containing high and low levels of In-As. The As contamination of these water sources originates from naturally occurring geological formations high in the Andes mountains. Arsenic levels of drinking water from both towns were originally identified through results of a prior study in the area (12). The high-exposure town, San Pedro (population 1600), has two sources of public water supply. The main source which serves most homes, comes from the Vila River and contains more than 600 μg As/liter. The second source of drinking water, the San Pedro River, has As levels around 170 μg/liter and is used by homes without piped water. We targeted primarily persons drinking Vila River water for this study.

The low-exposure town, Toconao (population 360), is located approximately 50 km from San Pedro. Water from the Jerez River, the main source of drinking water in Toconao, is piped into most homes and contains approximately 15 μg As/liter.

Announcements and public meetings were held describing the study. Local recruiters interviewed prospective participants to ascertain age, duration of residence, smoking status, and interest in participation. Recruitment was limited to those who were at least 18 years of age and had lived in the town for at least 2 months. Participants in both towns were frequency matched on age and smoking status. Recruitment continued until the desired sample size was reached.

We included only male participants in the study, because cells collected in male urine are almost exclusively transitional bladder cells, the target tissue of As-induced cancers (13, 14). Although females exfoliate a similar number of transitional cells as males, they also exfoliate squamous cells from the bladder trigone. Without the ability to differentiate squamous cells from transitional cells, any association between chronic ingestion of In-As and bladder cell MN in women would be diluted, biasing the results toward no effect.

Exposure Assessment and As Speciation. Each study participant was interviewed using a questionnaire regarding demographic variables, drinking water sources, fluid intake, dietary information, medical history, smoking status, occupation, and education. The first morning urine void was collected from each subject and analyzed for In-As and its urinary metabolites MMA and DMA.

The method of speciation used was a modified version of the method of Crecelius (15) and has been reported previously (16). In-As, MMA, and DMA were converted to their respective arsines by treatment with sodium borohydride under acidic conditions and were collected by sparging and cryogenic trapping. Following the collection of As vapors, the trap was allowed to warm and the arsine species were volatilized sequentially and detected by atomic absorption spectroscopy using a microburner combustion cell. Detection limits for each of the As species were 1–3 μg/liter, with coefficients of variation of 0.1–0.15.

In addition to dichotomous exposure status determined by residence in high- or low-exposure town, the sum of In-As plus the metabolites MMA and DMA (referred to as total urinary As) was also used to assess exposure, and individuals were divided into quintiles by their total urinary As levels. Urinary concentrations were also adjusted for creatinine; however, in the MN analyses, only unadjusted values were used, because they more closely reflect the As concentrations to which the urethelial cells were actually exposed. Moreover, results of a detailed exposure assessment analysis of this study revealed that the unadjusted urinary arsenic values correlated more closely with MN frequency than did the creatinine-adjusted values (17).

For urine collection, participants were supplied with precoded polypropylene bottles and instructions for urine collection. To obtain bladder cell samples, each subject was asked to provide a total of four urine samples in 2 consecutive days using only the second and third urine voids of the day. The first morning urine void was not used for exfoliated cell collection because exfoliated bladder cells tend to degrade from overnight exposure to urine. Bladder cells were isolated and stored as described previously (5).

Micronucleus Assay. Cells were permeabilized, the micronucleus assay was performed, and slides were scored as described previously (7, 18). In certain urine voids from some participants, cell pellets were contaminated heavily with crystals or a sand-like sedimentation that in some cases obscured cells on the slide. For such situations, we used 0.9% NaCl washes and a percoll gradient to separate the bladder cells from crystals without jeopardizing cell recovery.

Slides were coded and scored blindly in sets, each containing an equal proportion of slides from the high- and low-exposure groups. A total of 156 slides were obtained for 125 study participants. Of these, 105 slides contained exfoliated bladder cells for scoring. Up to 2726 normal cells were scored per person. Only cells that were not smeared, clumped, or overlapped and that contained intact nuclei were included in the analysis. Cells undergoing abnormal cell division and degenerative process such as karyorrhexis, karyolysis, nuclear fragmentation, and pyknosis were excluded from the analysis. However, we assessed the percentage of abnormal cells per person, which was calculated by scoring the number of cells with the degenerative processes of karyorrhexis and karyolysis in two sets of 100 cells each. If the two values differed by more than 15%, a third set of 100 cells was scored and incorporated into the analysis.

The prevalence of MNs was calculated based only on the number of intact, exfoliated cells using published scoring criteria (7). All questionable MNs were cross-checked by two observers and discussed until a consensus was reached. The presence or absence of cenotropic MS around each MN determined whether it arose via an aneuploidogenic (MN⁺) or a clastogenic (MN⁻) event. To determine whether MN were MN⁺ or MN⁻, probe staining in the MN was first compared to that in the main nucleus. Probe staining within the MN had to be bright and similar in structure and consistency to that
Table 1  Descriptive characteristics of study population in high- and low-exposure towns

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>High-exposure town (n = 70)</th>
<th>Low-exposure town (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>35.9 (20-75)</td>
<td>32.6 (20-70)</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>35 (50%)</td>
<td>30 (55%)</td>
</tr>
<tr>
<td>European</td>
<td>30 (43%)</td>
<td>25 (45%)</td>
</tr>
<tr>
<td>Other/Unknown</td>
<td>5 (7%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Mean yr residence (range)</td>
<td>19.3 (0.4-61)</td>
<td>28.3 (0.2-73)</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>25 (36%)</td>
<td>15 (27%)</td>
</tr>
<tr>
<td>Past smoker</td>
<td>20 (29%)</td>
<td>16 (29%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>23 (33%)</td>
<td>24 (44%)</td>
</tr>
<tr>
<td>Mean cigarettes/day (range)</td>
<td>1.6 (0-20)</td>
<td>0.9 (0-10)</td>
</tr>
<tr>
<td>Mean yr education (range)</td>
<td>7.7 (0-19)</td>
<td>8.2 (0-17)</td>
</tr>
</tbody>
</table>

Table 2  Summary of selected arsenic exposure variables: mean (range)

<table>
<thead>
<tr>
<th>Exposure index</th>
<th>High-exposure town (n = 70)</th>
<th>Low-exposure town (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water As (µg As/liter)</td>
<td>600 (360-670)</td>
<td>15 (13-17)</td>
</tr>
<tr>
<td>Tap water ingested (liters/day)</td>
<td>2.6 (0.07-7.3)</td>
<td>2.6 (1-6.0)</td>
</tr>
<tr>
<td>Urinary As (µg/liter)</td>
<td>616 (84-1933)</td>
<td>66 (4-267)</td>
</tr>
<tr>
<td>Adjusted urinary As (µg/liter)</td>
<td>588 (80-1234)</td>
<td>66 (7-305)</td>
</tr>
<tr>
<td>Urinary In-As (µg/liter)</td>
<td>185 (3-874)</td>
<td>100 (1-411)</td>
</tr>
<tr>
<td>Urinary MMA (µg/liter)</td>
<td>106 (4-329)</td>
<td>7 (1-23)</td>
</tr>
<tr>
<td>Urinary DMA (µg/liter)</td>
<td>391 (49-1223)</td>
<td>49 (1-245)</td>
</tr>
</tbody>
</table>

San Pedro River ranged from 134 to 170 µg As/liter, and those from Toconao ranged from 13-17 µg As/liter. We attempted to recruit only study participants drinking water from high (600 µg As/L) or low-exposure sources (15 µg As/liter), but a wide range of urinary As levels was found. Mean total urinary As levels were approximately 10-fold higher in the high-compared to the low-exposure group: 616 µg/liter (range, 84-1933 µg/liter) and 66 µg/liter (range, 4-267 µg/liter), respectively. In-As was 12-fold higher in the high-exposure group, and MMA and DMA were also much higher in the high-exposure group when compared with the low-exposure group (15-fold and 7.7-fold differences, respectively). Although the total urinary As levels in the high- and low-exposure groups differed greatly, the percentages of In-As, MMA, and DMA were similar (23). For example, in the high-exposure group, total urinary As contained 18% In-As, 15% MMA, and 67% DMA. In the low-exposure group, urinary As contained 16% In-As, 11% MMA, and 73% DMA.

Daily As exposure was also estimated from the questionnaire data (µg As/day) as well as daily As exposure relative to daily fluid consumption (µg As/liter fluid), enabling consideration that other fluids consumed may dilute the effect of As on the bladder (3). Whether water levels, urine levels, or questionnaire data were used to assess As exposure, it was clear that individuals from San Pedro are highly exposed compared to those in Toconao. However, 19 individuals from the low-exposure town had urinary As levels greater than 50 µg/liter, and of these, 4 individuals had urinary arsenic levels greater than 100 µg/liter. This variability was due in part to consuming water from the high-exposure town of San Pedro (i.e., if working or visiting), other water or dietary sources, or individual variations in drinking patterns or concentrations of urine. Therefore, a subset from the low-exposure town with background urinary arsenic levels (i.e., <50 µg As/liter) was identified for some of the analyses. Only two individuals from the high-exposure town had urinary As levels less than 100 µg/liter and both reported drinking water obtained from the low-exposure town.

A total of 66,355, 31,110, and 14,459 cells were scored in the high-, low-, and background-exposure groups. The corresponding average numbers of cells scored per person were 1005, 798, and 761. The prevalence of abnormal cells and the MNC/1000 cells in each exposure group are compared in Table 3. There was no difference between the prevalence of abnormal cells across the exposure groups. The prevalence of MNCs/1000 cells was 3.2/1000 cells in the high-exposure group as compared with 2.6/1000 cells in the low-exposure group, a 1.2-fold increase (95% CI, 0.95-1.6; P = 0.07). When the high-exposure group was compared to individuals with background urinary arsenic levels (<50 µg/liter), a 2.0-fold difference emerged (PR, 2.0; 95% CI 1.3-3.1; P < 0.001). We observed in the main nucleus. In cases in which probe staining in the main nucleus or MN was questionable, MN were automatically considered unscorable for the probe.

Statistical Analyses. The MNC prevalence for each group was calculated by dividing the total number of MNCs per group by the total number of cells scored for that group. This was done first for each town separately and then for each quintile of urinary arsenic for both towns combined. This ratio was expressed as a prevalence of MNCs per 1000 cells scored. The data were analyzed using STATA software (19). The PR of MNCs between groups was selected as the effect measure. This was computed by dividing the prevalence of MNCs in the higher-exposure group by that of the lower-exposure group. A Fisher’s exact test was performed to assess the differences in the prevalences of micronucleated bladder cells between exposure groups. Because it was hypothesized a priori that As exposure would be associated with an increase in the prevalence of MNCs, one-tailed tests were used (20). A Fisher’s exact test was also used to assess changes in the prevalence of centromere-specific MN frequencies found for each exposure group, and one-tailed tests were used, because it was hypothesized a priori that As exposure would be associated with an increase in the prevalences of both MN+ and MN on the basis of published in vitro findings. Exact 95% CIs were calculated (21). We also examined the effects of age and smoking as potential confounders and reexamined the effect of smoking for interaction.

To examine the dose-response relationships in quintiles 1–4, a trend test of proportions utilizing a 2xk contingency table was performed on the number of MNCs and on the MN+ and MN– MNCs (22).

Results

Descriptive characteristics of the study populations in the high (San Pedro)- and low (Toconao)-exposure towns are compared in Table 1. They were composed of 70 and 55 males, respectively, and were similar in age distribution, ethnicity, years of education, and smoking status. Years of residence were greater in the low-exposure town than in the high-exposure town: 28.3 and 19.3, respectively.

The mean and range of As exposure variables are presented in Table 2. Water samples collected from tap water in San Pedro (Vilama River water) between 1992 and 1994 ranged from 560 to 670 µg As/liter. Water samples collected from the
Table 3  MN measured in bladder cells in high and low As-exposed towns

<table>
<thead>
<tr>
<th>Exposure group (individuals*)</th>
<th>Cells scored</th>
<th>Urinary As μg/liter (range)</th>
<th>% abnormal cells</th>
<th>Prevalence of MNcs/1000 cells</th>
<th>PR*</th>
<th>95% CI*</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>High exposure (N = 65)</td>
<td>66,355</td>
<td>616 (84-1893)</td>
<td>39.2</td>
<td>3.2</td>
<td>2.0</td>
<td>1.3–3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low exposure (N = 39)</td>
<td>31,110</td>
<td>664 (4-2677)</td>
<td>39.8</td>
<td>2.6</td>
<td>NA'</td>
<td>NA'</td>
<td>NA'</td>
</tr>
<tr>
<td>Background As (&lt;50 μg/liter; N = 19)</td>
<td>14,459</td>
<td>32 (4-49)</td>
<td>39.3</td>
<td>1.6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Includes only individuals from whom exfoliated urothelial cells could be collected.

* The comparison presented is between the high-exposure group and those with background urinary arsenic levels (<50 μg/liter). Comparisons between the high- and low-exposure group are presented in the test.

* NA. not applicable. A comparison is not made between the low- and background-exposure groups, because the latter is a subset of the former.

Table 4  Centromere-specific probe MN prevalences in high and low As-exposed towns

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Prevalence of MN+&lt;1000 cells</th>
<th>PR*</th>
<th>95% CI* (P value*)</th>
<th>Prevalence of MN−&lt;1000 cells</th>
<th>PR*</th>
<th>95% CI* (P value*)</th>
<th>Proportion of MN+</th>
<th>Proportion of MN−</th>
</tr>
</thead>
<tbody>
<tr>
<td>High exposure</td>
<td>1.0</td>
<td>2.0</td>
<td>1.1–5.5 (0.02)</td>
<td>1.2</td>
<td>6.0</td>
<td>1.9–17.4 (0.001)</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Low exposure</td>
<td>0.8</td>
<td>NA'</td>
<td>NA'</td>
<td>0.7</td>
<td>NA'</td>
<td>NA'</td>
<td>1.4</td>
<td>NA'</td>
</tr>
<tr>
<td>Background As</td>
<td>(&lt;50 μg/liter)</td>
<td>0.5</td>
<td>NA</td>
<td>0.2</td>
<td>NA</td>
<td>NA</td>
<td>0.9</td>
<td>NA</td>
</tr>
</tbody>
</table>

* MN positive for the centromeric probe.

* The comparison presented is between the high-exposure group and those with background urinary arsenic levels (<50 μg/liter).

* Fisher's exact test (one-sided).

* MN negative for the centromeric probe.

* MNs, uncorrelated MN for which the mechanism of formation could not be determined.

* NA. not applicable. A comparison is not made between the low- and background-exposure groups, because the latter is a subset of the former.

Examined the effect of smoking and age as potential confounders and smoking for interaction; however, these factors did not alter the relationship between As and MN; therefore, only the summary associations are reported.

The urothelial cell centromere-specific probe prevalences (MN+, MN−) are presented in Table 4. When the high-exposure town was compared to the low-exposure town, the PR of MN+ was 1.3 (95% CI, 0.8–2.0). A larger 1.7-fold increase in the prevalence of MN− was also seen (95% CI, 1.1–2.7). Comparing the high-exposure group to the background-exposure group, greater increases in both MN+ and MN− were seen. The largest increase was seen in the prevalence of MN−, with a PR of 6.0 (95% CI, 1.9–17.4; P = 0.001), whereas the PR for MN+ was 2.0 (95% CI, 1.1–5.5; P = 0.02).

We also examined the difference in proportion of MN+ and MN− with respect to As exposure (Table 4). In the low-exposure group, MN− were less common than MN+, with 47% of MN− formed by chromosomal breakage and 53% by chromosomal lagging, respectively. In the background-exposure group, MN− were also less common than MN+, with 29% and 71% respectively. However, in the high-exposure group, MN− were more common than MN+, and chromosome breakage accounted for 55% of MN− formed.

In exfoliated cells exposed to a variety of genotoxic agents, severe genotoxic insult results frequently in the formation of multiple MN within a cell. This suggests that multiple MN are a good indicator of chemically induced genetic damage (25). In this study, a total of 13 multiple MNcs (5.4% of all MNcs) were found in the low-exposure group, and only 5 were found in the low-exposure group (1.1% of all micronucleate cells). No multiple MNcs were seen in the group with background levels of urinary As.

To determine the dose-response relationship between As exposure and MN formation, we divided the total study population into quintiles by total urinary As concentration (Table 5). The prevalence of abnormal cells increased from baseline (quintile 1) in quintiles 2 and 4, decreased in quintile 3, and did not change in quintile 5. The prevalence of MNcs/1000 cells showed a positive dose-response relationship with urinary arsenic in quintiles 1–4 (test for trend, P < 0.001). The MNc prevalence returned to baseline in quintile 5, when urinary As levels were >728.9 μg/liter (PR, 0.9; 95% CI, 0.6–1.6; P = 0.5).

To determine the mechanism of MN formation in each quintile, we characterized MN for the presence or absence of centromeric probe (Table 6). In quintile 1, 0.32 MN+<1000 and 0.26 MN−<1000 cells were found. In quintile 2, the prevalence of both MN+ and MN− increased significantly; however, the increase was greater in MN− than MN+: 4.7-fold (95% CI, 1.7–3.2; P = 0.001) and 2.3-fold (95% CI 1.0, 5.0, P = 0.03) respectively. In quintile 3, the prevalence of MN+ remained stable (1.04/1000 cells), whereas the prevalence of MN− increased to 7.5-fold over background (95% CI, 2.8–20.3; P = 0.001). In quintile 4, the prevalence of MN+ continued to increase to 1.57/1000 cells, a 3.1-fold increase over background (95% CI, 1.4–6.6; P = 0.002), whereas the prevalence of MN− decreased slightly to 1.36/1000 cells, a 5.2-fold increase over background levels (95% CI, 1.9–14.6; P = 0.006). The prevalence of both MN+ and MN−<1000 cells also showed a positive dose-response relationship with urinary arsenic in quintiles 1–4 (test for trend, P < 0.01 and P < 0.005, respectively). In quintile 5, in which urinary As levels were at their highest, the prevalence of both MN+ and MN− returned to background levels: 0.46/1000 and 0.25/1000 cells, respectively (95% CIs, 0.4–2.2 (P = 0.05), 0.6–2.3 (P = 0.6)).

Discussion

The results of this study provide additional evidence that chronic ingestion of In-As in drinking water is associated with an increased prevalence of MN in exfoliated bladder cells. Total urinary As was positively associated with the prevalence of MNcs in bladder cells in exposure quintiles 1–4 (urinary As levels between 4 and 729 μg/liter). This relationship was...
strengthened further by the observation that the majority of MN occurred primarily through chromosome breakage as shown by the PR of MN−. The prevalence of MN− increased as high as 7.5-fold (95% CI, 2.8−20.3; P < 0.001) in quintile 3, and the prevalence of MN+ increased to 3.1-fold in quintile 4 (95% CI, 1.4−6.6; P = 0.002). These findings are similar to results from the smaller North American MN study conducted by our group in which the frequency of MN containing acentric fragments (MN−) and those containing whole chromosomes (MN+) both increased [2.1-fold (P = 0.07) and 1.9-fold (P = 0.08; Ref. 25)].

It is noteworthy that in quintile 5, in which urinary As levels were highest, the prevalence of MN, MN+ and MN−per 1000 cells returned to baseline levels and proportions. This decrease is probably not indicative of a decrease in As genotoxicity but rather indicates that MN formation may be inhibited at high doses. There are several possible mechanisms by which MN formation may be inhibited. In vitro, similar dose-response relationships are observed frequently when the micro-nucleus and other cytogenetic assay are used to determine genotoxicity (26−28). Generally, the prevalence of MNCS/1000 cells is low at low concentrations, increases at higher concentrations, and decreases as concentrations of genotoxic chemicals reach cytotoxic levels. We attempted to use the percentage of abnormal cells as a measure of cytotoxicity to the bladder epithelium, but there was no relationship between As dose and abnormal cell prevalence. However, it is possible that this index may not be a reliable estimate of cell death in an exfoliated cell sample, because there are a number of ways in which dead exfoliated cells could escape the definition of “abnormal” cells (those with karyolysis or karyorrhexis). For example, cells missing large amounts of genetic material could die and lyse before reaching the tissue surface, and severely damaged cells may not withstand the mechanical steps involved in slide preparation and probe hybridization, causing the damaged cells to remain unobserved. In addition, sufficiently damaged “abnormal” cells that were misclassified as “unscorable” would remain uncounted, causing an underestimation of both cell death and the true cytotoxic effect of the agent involved.

Chemical exposure can also influence cell proliferation. Decreased cell proliferation can negatively influence MN prevalence, because (a) proliferation is needed to produce MN after genotoxic insult has taken place; (b) decreased cell proliferation could conceivably give cells more time for cellular repair (25, 29). Arsenic has been shown to increase the average lymphocyte proliferation time when lymphocytes from As-exposed and -unexposed populations were compared (30). Arsenic is also known to inhibit the growth of fingernails and toenails, producing transverse, white indentations called Mees’ or Aldrich Mee’s lines (31−33) as well as hair loss (34). It is possible that at the highest As exposures (quintile 5), cell division had been blocked so that severely damaged cells could repair themselves. Such chemically induced cell cycle arrest and subsequent DNA repair has been shown to occur as a normal cellular response to DNA damage by induction of the p53 gene (29).

It is noteworthy that this study did not find a relationship between age or cigarette smoking and the prevalence of micro-nucleated exfoliated bladder cells. This is consistent with other studies that also did not find a relationship between age and exfoliated cell MN prevalence (35, 36), however, unlike studies that have found a smoking-related increased frequency of micro-nucleated exfoliated cells of various origins (35−37). The apparent discrepancy may be due to the fact that in this study, smokers consumed on the average fewer than two cigarettes per day.

In conclusion, chromosome damage in the form of MN in human exfoliated bladder cells was shown to be induced by As exposure. The prevalences of MNCS/1000 cells increased in a dose-dependent manner at urinary As levels between 4 and
728.9 μg/liter. However, in the highest exposure group (individuals with urinary As levels >729 μg/liter), the prevalence of MNs/1000 cells returned to baseline, possibly due to increased cytotoxicity or cytostasis. Both factors would make cells less likely to express the genetic damage they have incurred in the form of MN. Predominantly centromere-negative MN were induced, which suggests that chromosome breakage was the major cause of MN formation; however, as also appeared to act as a weak aneuploidyogen. The relevance of the MNC biomarker to the increased bladder cancer risks for As ingestion remains to be elucidated and now requires a prospective study in an exposed population. However, these data show that As induces genetic damage in the form of chromosome breakage in the bladder urothelium at drinking water levels close to the current United States Maximum Contaminant Level for arsenic, which is currently 50 μg/liter.

References